Teacher Note – Scrape bacteria cells from plate directly into the 1.5mL micro centrifuge tubes for your students to use. Be sure to overload the water with bacteria cells. Also make sure you create a sample for your Williams water. Use the B-subtillus or the S-Marcenses depending on which one the teacher instructions tell you to use.

Module 1 – Isolation of DNA

1. Obtain 4 different bacteria in 1.5ml screw cap micro centrifuge tubes.
2. Centrifuge for 5 minutes to pellet the cells
3. Discard the supernatant – do not disturb the pellet
4. Add 100 microliters lysis buffer to the pellet
5. Tap to re-suspend the pellet (Vortex)
6. Put in 70 degree water bath for 15 minutes
7. Add 14 microliters of potassium acetate to each tube. Mix for 5 seconds. Ice for 5 minutes (Vortex)
8. Centrifuge for 5 minutes
9. Transfer supernatant to a .5ml micro-centrifuge tube (discard tube with the pellet after supernatant is saved)
10. Add 45 micro-liters of room temperature isopropanol to each tube. Centrifuge for 5 minutes.
11. Remove the supernatant (avoid losing the pellet). A small DNA pellet should be at the bottom of the tube. (DNA pellet is very small)
12. Wash the pellet with 20 micro-liters of cold 70% ethanol. Centrifuge 3 minutes
13. Discard the supernatant and allow the pellet to completely dry. (leave small amount of ETOH in tube)
14. Re-suspend the DNA in 25 micro-liters of Tris buffer.
15. Store tubes on ice until ready for module II.

Module II – PCR Amplification of the Multiplex Water Contaminants

1. Transfer PCR reaction pellet to the appropriate sized tube for the thermal cycler.
2. Label the tube containing the PCR reaction pellet with the sample and your initials
3. To each of your PCR pellet tubes add 20 micro-liters of primer mixture
4. Spin down DNA into pellets. Pipet off 100mL re-suspend in extra liquid.
5. To the first tube add 5 micro-liters of E-coli DNA
6. To the second tube add 5 micro-liters of B-subtilis DNA
7. To the third tube add 5 micro-liters of S-marcescens DNA
8. To the fourth tube add 5 micro-liters of Williams water DNA
9. Gently mix each of the tubes to collect the sample at the bottom. Make sure the PCR pellet is completely dissolved.

Positive Control

1. Transfer the PCR pellet to the appropriate sized tubes. Label the tube positive control.
2. Add 20 micro-liters of primer mixture
3. Add 5 micro-liters of E-coli positive control DNA.
4. Gently mix the tube to collect the sample at the bottom. Make sure the PCR pellet is completely dissolved.

Run the Thermal Cycler-

If 200bp ladder and PCR samples have been frozen heat for 2 minutes at 50 degrees – allow samples to cool. Load your gels.